REMARKS

Amendments

Claims 31 and 32 have been amended to specify that those cells expressing the positive or not expressing the negative markers are identified as cells with chondrocyte phenotypic stability.

Claim 31 has also been amended to require identifying markers codetectable with the positive markers BMP-2 and FGFR-3. Indeed, step (f) requires that the 'co-detectable markers' are identified based on the fact that they are co-expressed with the positive markers (BMP-2 and FGFR3) linked to the ability to form stable hyaline cartilage. Claim 31 has further been amended to remove the dash before "comprising" as well as the duplication of the words "to the". In addition, the definitive article "the" has been deleted from "the formation" and from "the in vivo formation."

In claim 32, the definitive article "the" has been deleted from "the formation" and from "the in vivo formed cartilage." Claim 32 has further been amended to insert the phrase "and/or specific reporter constructs comprising a promoter of said negative marker" (the term "negative" has been added to clarify the antecedent basis) after the phrase "said negative molecular marker."

In claim 34, "said set of positive markers" is replaced by "said positive marker." In addition, the word "a" has been removed between "in" and "cells from a cartilage biopsy."

In claim 36, the term "co detectable" has been replaced with "co-detectable".

New claim 60, which depends from claim 31, requires the use of ALK-1 as a negative marker in addition to a positive marker of chondrocyte phenotypic stability.

No new matter has been added by these amendments.

Applicants reserve the right to pursue any cancelled subject matter in this or a continuing application.

Claim objections

Claims 31 and 36 were objected to on several grounds. In view of the present claim amendments, these objections should be withdrawn.

Double patenting

Applicants hereby acknowledge the provisional obviousness-type double patenting rejection over various claims of copending Application No. 10/422,475, and agree to address this basis for rejection upon the indication of otherwise allowable subject matter in the present application.

Claim rejections under 35 U.S.C. § 112, second paragraph

Claims 31, 32, and 34 were rejected as indefinite under 35 U.S.C. § 112, second paragraph. As applied to the amended claims, these rejections should be withdrawn.

Claim rejections under 35 U.S.C. § 103(a)

Claims 31-36, 43-45, 51, and 55 stand rejected under 35 U.S.C. § 103(a) as unpatentable over Quarto et al. (herein referred to as "Quarto") in view of Binette et al. (*J. of Orthopaedic Research* 1998; herein referred to as "Binette") and Kolettas et al. (*Journal of Cell Science* 1995; herein referred to as "Kolettas"). Applicants note that claims 56 to 59 have not been included in this rejection. Accordingly, applicants undrstand that the rejection of the claims is limited to the extent that these claims refer to markers 'codetectable with BMP-2 or FGFR-3.' For the following reasons, this obviousness rejection, as applied to the amended claims, should be withdrawn.

Claims 31 and 32 have been amended to recite the identification of cells as having chondrocyte phenotypic stability based on the fact that they express BMP-2 and/or FGFR-3 and/or a marker co-detectable with these markers.

The Examiner states, on page 5 of the Office Action, that Quarto teaches a method of determining the expression of positive and negative markers of chondrocyte phenotypic stability using the steps of the present invention. Applicant strongly disagrees.

As previously stated, Quarto fails to observe stable, non-vascularized cartilage *in vivo*. Indeed, the Examiner acknowledges (on page 6, second paragraph) that Quarto does not even determine whether stable, non-vascularized cartilage is formed. It is noted that in fact under all conditions of cell expansion and implantation tested in Quarto vascularized tissue was formed together with cartilage by the implanted cells (see,e.g., Figure 6 and Table 1).

Quarto, on pages 4971 and 4972, describes the *in vivo* cartilage formation using two types of implants and a suspension of cells as a control. Importantly, the implantation of the suspension of cells leads to hypertrophic cartilage cells (i.e., cells which will turn into bone) showing infiltration with "a tissue histologically similar to hemopoeitic marrow." Accordingly, it cannot be stated that Quarto teaches or suggests a method including step (f) according to claim 31, wherein molecular markers are identified based on their co-expression with a positive marker linked to the formation of stable non-vascularized cartilage.

Moreover, Quarto uses adherent cells for the *in vivo* implantation assay. Results using the adherent cells are shown in Figures 3A1 and 3B1. Quarto provides no indication that the cartilage which is formed *in vivo* by these two populations differs. However the expression level of the different proteins shown in Figures 3A1 and 3B1 differs significantly between the two cell populations. As such, Quarto argues against any link between the nature of the cartilage formed *in vivo* and the expression pattern of the cells used for implantation.

Quarto further does not identify markers which are indicative of the ability to form stable non-vascularized cartilage. Instead, Quarto investigates the effect of different factors on the expression of collagen proteins and on the formation of cartilage *in vivo*. Indeed, the section of the Quarto publication (right column of page 4968) where collagens are determined relates to the *in vitro* differentiation of cells. Here collagen levels are determined for adherent cells at the start of the experiment (lane A1, B1, and C1 in Figure 3). The expression of these proteins is analyzed during the *in vitro* growth. The markers which are

determined are used to follow the differentiation of the cells.

Applicants moreover emphasize that even if Quarto had described 'a method to determine expression of markers' of chondrocytes, which is contested, this in itself is not the same as providing a method to identify cells having 'chondrocyte phenotypic stability', as presently required by the claims. Indeed, Quarto makes no statement about the fact that the expressed collagen proteins are markers or should be considered as indicative of production of cartilage *in vivo*. At most, Quarto uses the expression of collagen proteins to identify the chondrocyte differentiation pathway of the cells. There is however no indication or suggestion that these proteins are or can be used to identify the chondrocyte phenotypic stability of the cells, i.e. the ability of cells to make stable non-vascularized cartilage *in vivo*. In the absence of the observation of stable non-vascularized cartilage by Quarto, the Examiner's allegation that Quarto discloses the identification of collagen type II as a positive marker or collagen type X as a negative marker for chondrocyte phenotypic stability is inappropriate. On this basis alone, the obviousness rejection should be withdrawn.

Accordingly, based on Quarto, the skilled person is provided with a number of genes for which the association with stage of differentiation of the cells is provided. There is no indication of any link between the expression of these markers and the ability of the cells to form stable hyaline cartilage *in vivo*, nor of any method to determine this property of chondrocytes.

Neither Binette nor Kolettas remedy the deficiencies of Quarto. Indeed, Binette deals

with the fate of chondrocyte precursors in chondrocyte development. Chondrocyte precursors can become hypertrophic and ossify into bone. Alternatively, chondrocyte precursors can become articular chondrocytes. Again, Binette relates to chondrocyte differentiation and does not address the ability of chondrocytes to form stable non-vascularized cartilage *in vivo*.

The Examiner further asserts that the assessment of the extension of chondrocyte differentiation based on collagen type X expression by Binette is identical to the 'stability of the cells' phenotype' (page 7, lines 1-3 of the Office Action). Applicants disagree.

Binette does not investigate the ability of cells to produce stable hyaline cartilage *in vivo*. Binette, at most, demonstrates that collagen type X is not expressed by cells originating from articular cartilage. Like Quarto, there is neither a teaching nor a suggestion to use the identified marker in a method for assessing chondrocyte phenotypic stability, i.e. the ability of cells to produce stable hyaline cartilage *in vivo*.

The Examiner has asserted that Kolettas teaches that chondrocytes obtained from a cartilage biopsy express markers 'characteristic of cartilage'. Kolettas refers to the association of the expression of one or more cartilage-specific molecules with the loss of 'chondrocyte phenotype'. Kolettas' use of the term 'phenotype' in this article is best understood from the passage at the end of the left column on page 1997:

> *The morphological transition from a mesenchymal to a rounded phenotype using different culture conditions prompted us to investigate whether such phenotypic modulation is linked to changes in gene expression.*

Thus, the phenotype referred to in Kolettas relates to morphology and not to the ability to form hyaline cartilage *in vivo*. Kolettas' teaching therefore cannot be considered as indicative of 'chondrocyte phenotypic stability' in the context of the present invention.

Indeed Kolettas investigates which proteins have a different expression level when cells are passaged for prolonged time. Kolettas observed that after a prolonged period of time there is a morphological transition from a mesenchymal to a rounded phenotype, such that the cells no longer grow in monolayer (page 1997, left column, first paragraph). Kolettas found that chondrocytes cultured in monolayer express collagen type II and type IX collagen at the mRNA level. There is no indication that this is in any way associated with the ability of the cells to produce stable hyaline cartilage *in vivo*. Neither does Kolletas describe or suggest to use these markers as indicative of the ability of the cells to produce stable hyaline cartilage *in vivo*.

Summarizing, none of the documents either alone or in combination suggest a method for identifying cells capable of producing stable hyaline cartilage *in vivo*, making use of markers, let alone making use of BMP-2, FGFR-3 or a marker co-expressed with these markers.

Quarto describes an *in vivo* assay for determining cartilage formation but does not determine the expression profile of chondrocytes prior to the *in vivo* implantation, and in no way suggests that this expression profile could be indicative of the ability to produce stable hyaline cartilage *in vivo*. Kolettas and Binette describe different markers for differentiation

but nowhere investigate the ability of cells to produce stable hyaline cartilage *in vivo*, nor are they thus capable of identifying or suggesting a link between expression of these markers and the ability of cells to produce stable hyaline cartilage. Accordingly, it is submitted that the use of markers to identify cell populations of chondrocytes capable of producing stable hyaline cartilage in vivo, is not disclosed or suggested in any of the prior art documents, as presently recited in claim 31.

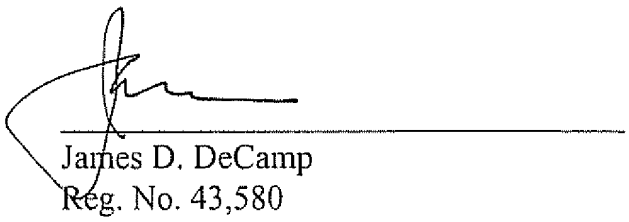
In view of the fact that the methods for identifying specific subpopulations of chondrocytes according to the present invention are not suggested in the cited references of record. Applicants submit that the skilled person would not have this method at his disposal for selecting these cells for transplanting cells to a connective tissue site in a patient or a method of seeding with cells any prosthetic device as claimed in claim 44 or for the provision of a therapeutic composition of claim 45. Thus the obviousness rejection against these claims should also be withdrawn.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is respectfully requested.

If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,



Date: 24 January 2007

James D. DeCamp
Reg. No. 43,580

Clark & Elbing LLP
101 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045